July 24th, 2016 – Plasmid Resuspension / Transformation of Plasmid

Objective: Resuspend synthesized plasmid, ensure circularity and then perform a transformation into DH5α E. coli cells on ampicillin

Results: Failed transformation, but DNA is in the medium ng/mL range.

August 9th, 2016 – Vector Transformation

Objective: Perform a transformation of our circularized plasmid into competent cells and then plate them on Amp plates.

Results: Successfully transform and obtain colonies on 2 of 4 plates. Plates placed in fridge for further use.

August 29th, 2016 – Heparin and Overnights

Objective: Weighed heparin into bottles for blood collection, then performed overnight cultures of our construct from the plates to obtain LB culture with our construct.

Results: Got 20 mg of heparin into bottles, along with cultures to work with the following day.

August 30th, 2016 – Miniprep

Objective: Perform a miniprep of overnight culture to obtain concentrated and purified plasmid DNA.

Results: Obtained 10 tubes of 50 uL DNA. Concentration will be determined later on.

September 1st, 2016 – Blood Observations

Objective: Examine blood and perform experiments involving its coagulation with overnight cultures of the construct.

Results: 1 of the 3 blood bottles was uncoagulated due to extra heparin addition. Blood experiments demonstrated that over time with our construct containing cells, blood clotted slightly faster than in comparison to control cells and without any cell addition at all.

September 5th, 2016 – Perform Multiple Digests

Objective: Perform cuts on all restriction sites individually. E, P, S and X.

Results: Will examine digest products on a gel but enzymes were enzymatically active based on lab mate usage.

September 12th, 2016 – DNA Digest Gel / Restriction DIgest

Objective: Run a 1% agarose gel of our restriction digest products. Showing that we had products in the lanes with E, P and S but not anything for the other lanes which included X and the backbone pSB1C3.

Results: Showing that we had products in the lanes with E, P and S but not anything for the other lanes which included X and the backbone pSB1C3.

September 14th, 2016 – Blood Work

Objective: Perform blood experiments with differing heparin, cell and control cell amounts.

Results: Showed that our construct clotted blood slightly faster, and that blood exposed to air and heparin could still clot after a short amount of time (2 hours).

September 21st, 2016 – Restriction of Backbone

Objective: E and P cuts on pSB1C3 backbone to obtain a vector that can be ligated into.

Results: Backbone was not present in a high enough concentration to be examined by gel.

September 28th, 2016 – Ligation

Objective: Perform digest followed directly by a ligation into the plasmid backbone. With the product of E and P digestions.

Results: Ligation was not successful but the backbone is funky.

October 3rd, 2016 – Canmore Digest and Gel/LB/Overnights

Objective: Ran 1% agarose gel, got overnights and LB media prepared.

Results: Canmore product shows two bands with differing weights. Overnight cultures made successfully for further use.

October 5th, 2016 – Periplasmic Prep

Objective: Create buffer solutions for periplasm prep.

Results: Sucrose buffer contained 50 mM Tris, 1 mM EDTA, 20% sucrose. Along with 5 mM $MgCl_2$ being created.

October 17th, 2016 – Periplasm Prep

Objective: Take 2 overnight cultures of our construct and perform the protocol on it.

Results: Final supernatant contained the results of the periplasm contents of our cells. To be run on SDS-gel to examine periplasm contents.